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Advances in science and technology: Evasion of the host immune response by pathogens

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Introduction

1. The topical scientific subject to be considered in 2014 is **"advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues"**. This is a particularly broad area covering a wide range of potentially relevant advances; however some developments reviewed under the standing agenda item on science and technology in the previous years of the current intersessional programme have implications also for our considerations this year. For example, in 2012 States Parties reviewed various enabling technologies, including those related to genomics and other '–omics', synthetic biology and systems biology, and agreed that their potential benefits to the Convention included, amongst other things, an improved understanding of the nature of disease and better healthcare technologies, such as improved, more efficient and economical vaccines, antibiotics and diagnostic systems. The United Kingdom paper on scientific and technological developments submitted for the Seventh Review Conference also highlighted that such technologies are useful tools in increasing the understanding of virulence mechanisms and host responses to pathogens and subsequently to development of new vaccines and therapeutics and improved diagnostics.[1] In the 2013 review, the advances recognised in relevant technologies for disease surveillance, detection, diagnosis and mitigation in many cases resulted from an increased understanding of the mechanisms of

[1] BWC/CONF.VII/INF.3/Add.1, pp 21-38.

disease and the host immunological response, as highlighted in the United Kingdom working paper on advances in vaccine development.[2]

2. Previous reviews also considered particular implications of such advances for the BTWC, including the potential for uses contrary to the provisions of the Convention, some of which will also be relevant to this year's topic; for example, increased capacity to manipulate pathogenicity, host specificity, transmissibility, resistance to drugs, or ability to overcome host immunity. In this context, States Parties have recognised that it is important to consider appropriate oversight measures to identify and manage associated risks in a way that will maximise the benefits of advances while minimising the risk of their application for prohibited purposes. The potential implications of advances in the understanding of pathogenicity, virulence, toxicology and immunology for other provisions of the Convention can also be recognised. These include those related to dealing with the consequences of a violation of the Convention, both in providing assistance to a State Party affected as a result and in any investigation process; and those relevant for capacity building and cooperation in the prevention of disease or for other peaceful purposes.

3. Given the immense range of potentially significant advances and the relevance of some topics previously reviewed, this Working Paper focuses only on one particular aspect: advances in the understanding of the strategies developed by pathogens to evade the host immune response and the consequent implications for the BTWC.

Evasion of host immune response: Background

4. In order for a microorganism to cause disease in a host organism, it must develop strategies to overcome the host mechanisms to defend against infection. Successful microbial pathogens have evolved strategies to evade both innate and adaptive immune systems[3], which may involve avoiding recognition by the immune system, interfering with its function, or degrading its components. There are many published reviews on this topic some of which outline general mechanisms used to subvert and exploit immune systems by diverse microbial pathogens,[4] and others which address mechanisms that have evolved in specific types of pathogens.[5] A fundamental expectation of studies in this field is that increased understanding of the molecular details of host-pathogen interactions and anti-immune systems will lead to a better understanding of the weaknesses in host defence systems and assist the development of vaccines and therapeutics. The following section provides a few recently published examples which reflect some of the different types of strategies utilised by pathogens in evasion of the host immune response.

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[2] BWC/MSP/3013/MX/WP.8.

[3] The innate immune system is the first line of host defence against invading pathogens and comprises cells and mechanisms that defend the host from infection in a non-specific manner, and activate the adaptive immune system. The adaptive immune system is specific to the invading pathogen and can provide long-lasting immunity.

[4] For example, Finlay, B.B & McFadden, G (2006) Anti-Immunology: Evasion of the Host Immune System by Bacterial and Viral Pathogens. Cell 124: 767-782.

[5] For example, Higgins, M.K & Carrington, M (2014) Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. Protein Science 23: 354-365; and Smith et al (2013) Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. Journal of General Virology 94: 2367-2392.

Evasion of host immune response: Some examples

5. Dengue virus has developed an ability to hide from host immune defences by avoiding recognition by components of the innate immune system. To do so it requires an enzyme known as MTase (or 2'-O-methyltransferase) to chemically modify its genetic material and escape detection, and thus survive, replicate and cause disease. Increased understanding of this mechanism has assisted progress towards effective vaccines and antivirals. For example, a recent study has reported a novel strategy in dengue vaccine development based on attenuated virus strains with a genetic mutation introduced to deactivate the MTase enzyme.[6] In these strains, the unmodified genetic material is immediately recognised by the host and triggers a response by innate immune factors. The viruses also elicit a strong adaptive immune response conferring protection on the host. Furthermore, it was found that that the MTase mutant dengue virus cannot infect the mosquito vector responsible for its transmission. This proof-of-concept study demonstrated that the attenuated viruses were stable, safe and immunogenic, and showed their potential as a safe, highly immunogenic, rationally-designed dengue vaccine approach.

6. Severe acute respiratory syndrome coronavirus (SARS-CoV) uses a different strategy to avoid detection in the host. It produces an enzyme called PLpro (or papain-like protease) which removes host cell proteins involved in triggering the innate immune response. This interferes with the host cell's signalling pathways and prevents it from alerting the immune system to the presence of the virus, thus allowing it to survive and replicate undetected. Increasing the understanding of such a mechanism will be of critical importance in providing a strategy for the development of vaccines and antivirals against SARS-CoV and other coronaviruses, such as MERS-CoV, for which there are currently no approved countermeasures. However, PLpro has another key function essential for viral replication, which has to be retained in a live vaccine strain to allow sufficient viral particles to be generated to trigger the immune response. Thus the goal of rational vaccine design would be to knock-out the system for evasion of the immune response whilst retaining the ability to replicate in host cells. A recent effort to understand the structural basis of PLpro activity has revealed key sites in the enzyme required for recognition and processing of the host cell proteins and elaborated its role in antagonism of the innate immune response.[7] This information enabled the creation of mutations in the enzyme to prevent interaction with the host cell proteins, while retaining the function for viral replication. Further work in this area is required, but this may represent a first step towards the development of a safe, live attenuated vaccine.

[6] Züst et al (2013) Rational design of a live attenuated dengue vaccine: 2'-O-methyltransferase mutants are highly attenuated and immunogenic in mice and macaques. PLoS Pathogens 9(8): e1003521. doi:10.1371/journalppat.1003521.

[7] Ratia et al (2014) Structural basis for the ubiquitin-linkage specificity and delSGylating activity of SARS-CoV papain-like protease. PLoS Pathogens 10(5): e1004113. doi:10.1371/journal.ppat.1004113.

7. Some pathogenic bacteria have an outer protective layer formed of capsular polysaccharides, which prevents them being recognised and destroyed by the host immune system. Thus the disruption of this layer could provide a strategy for therapeutic development, as highlighted in a recent publication.[8] This study focused on *Escherichia coli*, which forms the capsular polysaccharide inside the cell, then transports it through a membrane pore to the cell surface. The strategy involved using compounds that resembled the exported polysaccharide to block the pore and therefore disrupt formation of the protective outer layer. This allowed the bacteria to be recognised and killed by the host immune system, and hence could be key to the development of a new antibiotic class active against pathogens with similar mechanisms for capsule formation.

8. Some viral, bacterial, fungal and parasitic pathogens use a mechanism known as antigenic variation to avoid recognition by the adaptive immune response. The adaptive immune response relies on memory of previous exposure to specific antigens, which stimulated the generation of specific antibodies to target those antigens. Thus changing the molecules involved in promoting that response can circumvent the clearance of subsequent infection or sustain an on-going infection.[9] One of the most well-known and well-studied examples is influenza virus, where continuing to increase the understanding of the evolutionary patterns and molecular basis of antigenic change is important for vaccination and surveillance considerations. Recently published findings from research on the bacterium *Helicobacter pylori* (which causes ulcers in humans) suggest that it uses a strategy of rapid mutation during the initial infection phase that allows it to evade the immune system by quickly evolving. This results in elevated frequencies of changes in its surface proteins which may no longer be recognised by antibodies, thus allowing the bacteria to avoid the host immune response and to establish a chronic infection.[10] Trypanosomes and *Plasmodium* species, parasitic pathogens that cause sleeping sickness and malaria respectively, despite having very different life cycles have both evolved mechanisms to switch expression of variant surface proteins through antigenic variation to avoid antibody binding. Further studies will help understanding of the molecular details of the host-parasite interactions and guide the development of therapeutics to tackle parasitic disease.[11]

9. A key element of the innate immune response is the recognition of microbial components by receptors on the surface of host immune cells. When pathogens invade the host, proteins known as Toll-like receptors (TLRs) recognise pathogen-associated molecular patterns (PAMPs) and trigger a signalling pathway to activate an immune response. Within TLRs there is a conserved region known as the TIR domain which is critical for the protein-protein interactions involved in the signalling pathway. Recent work has identified a potentially novel evasion mechanism involving bacterial TIR domain proteins in a wide range of pathogenic bacteria, including *Salmonella enterica*, *Brucella*

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[8] Kong et al (2013) Single-molecule interrogation of a bacterial sugar transporter allows the discovery of an extracellular inhibitor. Nature Chemistry 5: 651-659.

[9] For review, see for example, Kotwal G. J & Kulkarni A.P (2013): Antigenic variation in microbial evasion of immune responses. eLS: doi: 10.1002/9780470015902.a0001207.pub3; and Lipsitch M & O'Hagan J.J (2007) Patterns of antigenic diversity and the mechanisms that maintain them. Journal of the Royal Society Interface **5**: 787-802.

[10] Linz et al (2014) A mutation burst during the acute phase of *Helicobacter pylori* infection in humans and rhesus macaques. Nature Communications 5: doi: 10.1038/ncomms5165.

[11] Higgins, M.K & Carrington, M (2014) Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. Protein Science 23: 354-365.

sp., E.coli and *Yersinia pestis*.[12] There is evidence to suggest that these interfere directly with the TLR signalling pathway and thus inhibit the activation of the innate immune system. Further understanding of the modes of action and the roles in virulence of these domains may help in developing strategies for novel countermeasures.

10. A further example of an evasion system involving interaction with receptors on the surface of host immune cells is a mechanism by which viruses such as influenza, West Nile and dengue activate a class of molecules, known as TAM receptors, which are central inhibitors of the innate immune response to pathogens. A recent study reported that a substance called phosphatidyl serine, found on the surface of enveloped viruses, bound to the immune cell surface proteins leading to disablement of the innate immune response.[13] Understanding of this mechanism may provide an attractive target for novel antiviral therapies which could block the virus's ability to activate TAM receptors and thus prevent interference with the innate immune system.

11. *Yersinia pestis* (the causative agent of plague) also disrupts the host immune system by interacting with a receptor protein on immune cells. *Y.pestis* requires the presence of a protein called the plasminogen activator protease (Pla) to survive within the lungs. It has recently been demonstrated that Pla activity degrades a molecule known as the Fas ligand (FasL) which is involved in the signalling process for a form of programmed cell death known as apoptosis. This interferes with the innate immune system and alters the host inflammatory response, enabling enhanced bacterial growth in the lungs, thus facilitating infection.[14] Pneumonic plague is fatal if untreated, and antibiotics are effective only if administered early, within the first 24 hours. Further understanding of the process by which *Y.pestis* evades the immune response may help in the development of effective medical countermeasures, for example by exploring the possibility that blocking Pla activity and thus restoring FasL signalling might give antibiotics more time to work.

12. A mechanism common to many bacteria involves the hijacking of host cells by the injection of microbial proteins in a so-called Type III secretion process, by which they exert a number of effects that assist their survival and block the host cell immune response. In some pathogens, one effect is to exploit the actin[15] polymerisation processes of the host cells to acquire actin tails that allow the bacteria to traffic rapidly through the host cell and from cell to cell.[16] Rapid cell-to-cell spread facilitates the avoidance of host innate and adaptive immune responses. This type of mechanism could provide a target for the development of antimicrobials with a novel mode of action, but further investigation is required to understand better the molecular processes involved.

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[12] See for example, Rana et al (2013) Bacterial TIR-containing proteins and host innate immune system evasion. Medical Microbiology & Immunology 202: 1-10; and Salcedo et al (2013) BtpB, a novel *Brucella* TIR-containing effector protein with immune modulatory functions. Frontiers in Cellular and Infection Microbiology 3: 1-13.

[13] Bhattacharyya et al (2013) Enveloped Viruses Disable Innate Immune Responses in Dendritic Cells by Direct Activation of TAM Receptors. Cell Host & Microbe 14: 136-147.

[14] Caulfield et al (2014) The Pla protease of *Yersinia pestis* degrades Fas ligand to manipulate host cell death and inflammation. Cell Host & Microbe 15: 424-434.

[15] Actin is a cellular protein involved in muscular contraction, cell motility and maintenance of cell shape.

[16] Allwood et al (2011) Strategies for intracellular survival of *Burkholderia pseudomallei*. Frontiers in Microbiology 2: 1-19.

13. A possible mechanism to evade the adaptive immune response that has recently been reported involves bacterial production of a broadly-reactive antibody-binding protein.[17] The protein, discovered in a *Mycoplasma* species that infects humans, was found to bind with high affinity to all types of human and non-human antibodies through attachment to conserved regions, thus blocking antibody-antigen binding. Since the interaction of specific antibodies with invading pathogens plays a key role in preventing infection, this represents an effective evasion strategy that interferes with the adaptive immune response. However further studies are required to elucidate this potential mechanism to defeat the antibody response, which if confirmed may offer a target for new antibacterial therapies. Given its apparent ability for broad-scope, high affinity binding to antibodies, another potential use for this protein is in the large scale purification of therapeutic antibodies.

14. Another mechanism recently described involves the degradation of a metabolite produced by immune cells called macrophages. These cells engulf invading pathogens inducing the production of a chemical called itaconate which interferes with bacterial metabolism. However some pathogens, including *Yersinia pestis,* can degrade itaconate, and three genes have been found to encode the enzymes responsible; these are crucial for the survival of some pathogens in macrophages.[18] In this case, as well as neutralising host defence mechanisms, the strategy also aids survival in the macrophage by breaking down itaconate into components that provide nutrients for the pathogen. The enzymes involved in this process might provide potential targets for the development of new antimicrobial agents.

Implications and conclusions

15. The above recent examples represent just a small proportion of the vast quantity of research on evasion of the host immune response by pathogens. The amount of effort in this area reflects its importance in the campaign to discover and develop novel strategies to address the challenges in defeating infectious disease. However, as with many fields in the life sciences, advances on this topic have the potential for uses contrary to the provisions of the Convention. Knowledge gained through research on host-pathogen interactions and mechanisms used to overcome the host immune response could also be exploited for harmful purposes, for example in designing novel biological weapons agents or engineering existing agents to increase their suitability for biological weapons use. It is thus important to ensure that appropriate oversight and governance strategies for such dual-use research are in place to minimise the risk of its use for prohibited purposes, without having adverse effects on crucial progress on infectious disease control. It is clear from recent discussions and debates on dual-use research issues that it is critical to undertake consideration of all the implications of proposed work, including those related to publication, at an early stage to ensure that both the potential benefits and risks are clearly balanced and articulated.

16. Advances in the understanding of evasion of the host immune response by pathogens have wide reaching potential benefits for the mitigation of infectious diseases and are highly relevant for the BTWC. The application of discoveries in this field to the development of vaccines and therapeutics has key implications for progress on the global response to infectious disease outbreaks, whether natural, accidental or deliberate. This is of relevance to the strengthening of Article VII, in providing assistance to any State Party exposed to danger as a result of a violation of the Convention, the biennial topic being

[17] Grover et al (2014) A structurally distinct human mycoplasma protein that generically blocks-antigen-antibody union. Science 343: 656-661.

[18] Sasikaran et al (2014) Bacterial itaconate degradation promotes pathogenicity. Nature Chemical Biology 10: 371-377.

addressed by States Parties in the remaining two years of the current intersessional programme. It is also a significant issue for activities related to Article X, in the development and application of scientific discoveries for the global prevention of disease, and for other peaceful purposes, and is thus relevant to the standing agenda item on cooperation and assistance.

17. The breadth of this year's topic again underlines the benefits of bringing in a range of knowledge and expertise from those more closely involved in relevant areas to assist us in considering the implications across all aspects of the Convention, and in assessing the need for effective action to manage potential benefits and risks. It also highlights the cross-over between the topics addressed in the review of scientific and technological advances over the years of the current intersessional programme, and thus the need to consider implications from previous understandings in our continuing reviews.